are complex and may differ among tissue-specific isoforms nevertheless, mechanistic understanding of the molecular regulation of the channel is beginning to emerge. We and others have demonstrated the importance of the carboxyl terminus (CT) in the regulation of the channel. The CT is a hot spot for mutations that produce inherited cardiac arrhythmias, myotonias, epilepsy and autism. In the case of NaV1.5, the cardiac channel, mutations of critical structural motifs in the CT (including an EF hand-like motif and an IQ motif) result in disease conditions such as Brugada and LQT syndromes. Also, altered NaV channel trafficking and function with consequent intracellular Na+ overload contributes to the development of dilated cardiomyopathy. The structure of the CT of the Nav1.5 channel in complex with calmodulin (CaM), determined to 2.9 Å resolution, shows that many of the mutations associated with disease states occur at CTNav1.5-CaM interfaces. Based on this structure a mechanism for the transition to the non-inactivated state of the channel is proposed.

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Calmodulation of Voltage-Gated Calcium Channels by Blue Light

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Delivering custom-shaped signaling inputs to dissect biological networks has been a coveted goal of engineering-minded biologists, keenly aware of the advantages of analyzing electronic circuitry with a signal generator. We have recently devised a rapamycin-based chemical-dimerization strategy to produce step-like increases in the concentration of calmodulin (CaM) at the cytoplasmic mouth of L-type Ca^{2+} channels. This tactic revealed that CaM binding to channels induces two effects: a large increase in peak open probability (P_O), and the ability to undergo Ca^{2+}-dependent inactivation (CDI) (Adams *et al* (2014) *Cell*, **in press**). Yet, the rapamycin system is comparatively slow ($t_{rise\text{-}half}$ ~30 s) and irreversible. Here we explore the use of a light-based dimerization system from *Arabidopsis* (CRY2 and CIB1, Kennedy *et al* (2010)) to deliver CaM more rapidly and reversibly upon activation by blue light. To evaluate functionality with Ca^{2+} channels, HEK293 cells expressing Ltype Cav1.3 channels were endowed with CIB1 targeted to the membrane and CRY2 glycine linked to a dominant-negative CaM_{1234}, where mutations eliminate Ca^{2+} binding and presumably render this construct a constitutive apoCaM. On dimerization with blue light, CDI was indeed attenuated, confirming functionality with Ca^{2+} channels. More telling, blue-light exposure left peak currents unchanged, arguing that CaM_{1234} in fact acts just like apoCaM. Next, we expressed a variant of $Ca_V1.3$ (MQDY) with diminished apoCaM binding at baseline, and changed the CIB1 payload to wild-type CaM. Reassuringly, blue-light exposure increased both peak current and CDI by ~20% (5/6 responding cells), consistent with enhanced MQDY binding to CaM upon blue-light recruitment. Intriguingly, full enhancement occured in the very first current evoked after blue-light illumination (<20 s); CaM exchange with these channels thereby occurs on an even faster timescale. In all, light-based recruitment promises a powerful tool for investigating CaM modulation of membrane proteins.

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Pegylated Cholesterol and Methyl-Beta-Cyclodextrin are Modulators of L-Type Calcium Channel Current and Decrease Membrane Capacitance in Vascular Smooth Muscle Cells

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Loading of pegylated cholesterol (PEG-cholesterol) by pretreatment of several hours decreases current density and augments voltage-dependent inactivation of L-type Ca channel current (ICa,L) and depletion of cholesterol by similar long pretreatment with methyl-beta-cyclodextrin (MbCD) increases the ICa,L density in A7r5 aortic smooth muscle cells. On the other hand, dehydroepiandrosterone (DHEA), a cholesterol-derived steroid hormone, rapidly induces voltage-dependent inhibition of ICa,L. If endocytosis and exocytosis of CaV1.2-containing membrane vesicles are involved in these modulations, they should induce a change in membrane capacitance (Cm). Cm could be affected also by a change of thickness and dielectric constant of lipid bilayer. Here we studied acute effects of PEG-cholesterol, MbCD and DHEA on Cm of isolated bovine coronary artery smooth muscle cells by whole-cell patch clamp technique. Ramp steps of 5 or 10 ms were applied repetitively before, during and after application of the modulators. Control Cm was 18.3 ± 4.5 pF (mean ± SD.). PEG-cholesterol and MbCD induced gradual and small decrease of Cm and their washout decelerated the rate of the decrease. Cm after 10 min of wash-in normalized by the initial value was: control without modulator, 1.01 ± 0.02 (mean ± SD.); 1 mM PEG-cholesterol, 0.96 ± 0.02 ($p<0.01$, compared with control); 10 mM MbCD, 0.90 ± 0.05 ($p<0.0001$); 0.1 mM DHEA, 1.00 ± 0.03. Since the change of Cm is small, it little affects qualitative conclusion of the modulation of ICa,L density obtained by the pretreatment experiments with PEG-cholesterol and MbCD. The small but significant decrease of Cm reflects structural changes of the lipid bilayer which may be involved in the modulation of ICa,L by PEG-cholesterol and MbCD.

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NMDA Receptor Inhibition of L-Type Calcium Channels via ER Calcium Depletion and Activation of STIM1 in Cultured Hippocampal Neurons

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We have found that, in cultured hippocampal neurons, NMDA receptors (NMDAR) inhibit voltage-gated L-type Ca^{2+} channels through Ca^{2+} release from the endoplasmic reticulum (ER) and subsequent engagement of STIM1 with L channels. Here we employed laser spot photo-uncaging of glutamate near single dendritic spines combined with fluorescence Ca^{2+} imaging to investigate the relationships between stimulus frequency (glutamate uncaging) and Ca^{2+} release from the ER. Measurements of cytosolic Ca^{2+} were made with the genetically-encoded Ca^{2+} indicator, RGECO1, and simultaneously, measurements of $[Ca^{2+}]_{ER}$ made with a genetically-encoded Ca^{2+} indicator targeted to the ER, D1ER. Using a laser pulse duration of 2 ms to photo-uncage glutamate near a single spine, simultaneous measurements of $Ca^{2+}{}_{cyto}$ and $[Ca^{2+}]_{ER}$ revealed that stimulation at 1 second intervals (1 Hz) for 60 seconds triggered a rapid rise in $Ca^{2+}{}_{cyto}$ followed by release of Ca^{2+} from the ER. Spine stimulation at 6 second intervals (0.167 Hz) for 60 seconds elicited a large cytosolic Ca^{2+} transient, but no significant Ca^{2+} release from ER stores. At various stimulus frequencies, pharmacological analysis using the L-type Ca^{2+} channel blocker, nimodipine, uncovered a direct correlation between the magnitude of the nimodipine-sensitive, L-channel Ca^{2+} transient and Ca^{2+} release from the ER. Together, these results suggest that even though the lowest frequency stimulation is capable of generating a cytosolic Ca^{2+} signal, the NMDAR Ca^{2+} signal must contain the necessary details to activate L-channels by membrane depolarization and integrate with the L-channel Ca^{2+} signal to regulate Ca^{2+}-induced Ca^{2+} release in a single dendritic spine. If this frequency dependence holds true for the activation of STIM1 to regulate L-channel, this work will have important implication in how we think about channel regulation.

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Defining Post as a Modulator of STIM1 Function during T Cell Activation

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Upon engagement of the T cell receptor, InsP3 is produced, triggering the release of Ca^{2+} from ER Ca^{2+} stores. The resulting ER Ca^{2+} store depletion is sensed by STIM1, which relocalizes to ER-plasma membrane junctions to activate Ca^{2+} channels and inhibit Plasma Membrane Ca^{2+}/ATPase (PMCA)-mediated Ca^{2+} extrusion, thereby enabling sustained increases in cytosolic Ca^{2+} that drive the process of T cell activation. Partner of STIM1 (POST) is a recently identified STIM1 adaptor protein with multiple binding partners, including STIM1, PMCA and several other transporters, pumps and exchangers. POST is a multi-pass transmembrane protein found in the ER and PM predicted to have 10 transmembrane domains. To assess this predicted topology, we used fluorescence protease protection assays, finding that POST is actually a 9 transmembrane-containing protein with a cytosolic N-terminus and a luminal/extracellular C-terminus. To assess the dynamics of STIM1/POST-mediated control of PMCA activity, we utilized colocalization and FRET. Upon T cell activation, POST migrated and co-localized with both STIM1 and PMCA4 to the immunological synapse. Interestingly, FRET between POST and both proteins was observed, yet FRET between STIM1 and PMCA4 could not be detected. Finally, POST knockdown inhibited activation-dependent inhibition of PMCA4-mediated Ca^{2+} clearance. These studies provide new insight into the topology and dynamics of POST/STIM1/PMCA4 interactions during T cell activation. Given its numerous targets, further investigations into POST function may reveal additional roles for POST in the T cell activation process.

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The Sigma1 Receptor Competes with STIM1 to Bind Orai1 to Regulate Store Operated Calcium Entry (SOCE)

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The Sigma1 receptor is an ER chaperone protein targeted to mitochondrial associated ER membrane regions (MAMs). An increase in its expression